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THE RELATION OF NUMBERS OF ANIMALS TO SURVIVAL IN TOXIC CONCENTRATIONS OF ELECTROLYTES

A DISSERTATION SUBMITTED TO THE GRADUATE FACULTY IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

By
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THE RELATION OF NUMBERS OF ANIMALS TO SURVIVAL IN TOXIC CONCENTRATIONS OF ELECTROLYTES[1]

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I. INTRODUCTION

THE harmful effects of crowding animals into a limited environment have long been known. The literature on this subject is too extensive for detailed review at this time. The development of the present-day information and point of view may be traced briefly as follows: Hogg (1854) explained such harmful effects among snails as due to their ability to adapt themselves to the necessities of their environment. Semper (1874) thought the ill effects of crowding were due to a lack of sufficient growth-producing substance in small volumes of water. Yung (1878) explained them among tadpoles as due to a lack of aëration. De Varigny (1894) concluded that a lack of exercise was the causal factor for dwarfing among snails. Vernon (1895), using echinoderm larvae; Warren (1900), using *Daphnia;* Woodruff (1911, 1914), using different Protozoa, all concluded that these harmful effects were due to accumulation of waste products in small volumes. Colton (1908) thought food and oxygen as well as waste products were influencing factors among snails. Bilski (1921) concluded that these effects among tadpoles were caused by excessive expenditure of energy on account of contact stimulations; Goetsch (1924) confirmed these results for rapidly moving animals. Pearl and Surface (1909) suggest a psychological factor to explain crowding phenomena among chickens. Peebles (1929), using sea-urchin and starfish larvae, found evidence of a growth-inhibiting substance which is of a lipoid nature

[1] I wish to express my sincere thanks to Dr. W. C. Allee for suggesting this problem and for his kindly counsel throughout the work upon it. I also wish to express my thanks to Dr. Sewall Wright for valuable advice as to statistical treatment of the data.

and soluble in acetone. This literature is reviewed in greater detail by Allee (1931).

Drzewina and Bohn (1921*c*) found that *Polycelis* did not survive as long in groups as they did as single individuals when placed in 0.03 N. KCl solution. They postulated an autodestructive secretion to account for this. Later (1925) they explained this autodestructive effect as being due to low pH brought about by cell cytolysis which accelerates further cell cytolysis.

The beneficial effects of crowding a number of animals into a limited volume when the environment is uncongenial was overlooked until about ten years ago, when Allee (1920) reported that land isopods, water isopods, and brittle starfish, when aggregated, respired more slowly and remained quieter, and under extreme conditions survived longer, than single individuals in the same environment. Drzewina and Bohn later in the sameh year published results whic show that *Convoluta*, a marine flat worm, will survive much longer as groups than as single individuals when placed in dilute sea water. Since that time, there has accumulated considerable literature on this subject, which on the whole shows that, other conditions being equal, groups of animals may have a marked advantage in survival time over single individuals when placed in small volumes of toxic media. There have been several theories advanced at different times and under different conditions to account for this group protection against toxic media.

Drzewina and Bohn (1921*a*, *b*, *c*), experimenting with Infusoria, *Hydra*, *Convoluta*, and tadpoles of *Rana fusca* in toxic solutions of colloidal silver, potassium chloride, and fresh water, found markedly increased survival when animals were grouped over that of single individuals when exposed to the same concentration and the same volume of the toxic solution. In order to explain this phenomenon, they postulated an autoprotective secretion which they suggested is a substance (or substances) secreted by the animals which destroys the deleterious effects of the toxic solution.

Bresslau (1922, 1924) studied the effects of different dyes—methylene blue, Victorian blue, neutral red, India ink, etc.—on different Infusoria, such as *Colpidium*, *Paramecium*, etc., and found that groups of animals in a small volume of the weak dye solution sur-

vived much better than single individuals in the same volume and concentration. He accounted for this protective value of the group by the fact that these animals secrete a substance, which he calls "tektin," that forms a gelatinous hull around them and adsorbs the toxic chemical, thereby weakening its concentration and making it possible for groups to live longer than single individuals.

Allee and Schuett (1927) experimented with *Planaria* and other fresh-water animals and with *Ophioderma* in toxic solutions of colloidal silver and found marked group protection. They explained their results on the basis that these animals secrete a slime which adsorbed the silver and weakened the concentration of the medium, thus permitting the groups to survive much longer than single individuals. Their results show that this protection is not specific in effect.

Allee (1927*a*) experimented with *Ophioderma* and found that groups survived better than single individuals when under adverse laboratory conditions. When glass rods were placed in the vessel with single individuals, the individuals survived better than other individuals in the absence of glass rods. He concluded that the survival advantage of the group was at least partly due to members of the group substituting each other for objects of the normal environment, which regulated the general body tonus and made it possible for the groups to survive better than single individuals.

Allee (1929*a*) experimented with *Procerodes* in hypotonic sea water in which the electrolytes were controlled, and found that when the hypotonic sea water was conditioned with extracts of the same and of different species the animals survived significantly longer than they did in unconditioned hypotonic sea water with the same strength of electrolytes.

Drzewina and Bohn (1928), in their summarizing paper, "Les *Convoluta*," review the literature on the subject of mass protection and recognize the validity of the foregoing theories under different conditions and add another theory, which they call "catalysis by contact." This is an expression of the changes in electrical conditions produced by a group, as contrasted with those found when single animals are isolated, and is concerned with interrelations between animals as well as relations between individuals and the walls of their container.

For an interpretation of our data and an understanding of the point of view developed in the present paper, attention also needs to be called to the work of Child and his collaborators, who have repeatedly demonstrated that the animal or part of an animal which has the highest metabolic rate is the most susceptible to a great variety of injurious agents, both chemical and physical. They have shown this to be true for a large variety of animals and plants. Also, if these injurious agents are not too fatal, so that there is some acclimation, the organism or part of the organism with the highest metabolic rate will acclimate most rapidly and survive longer than one with slower metabolic rate. He calls the former "differential susceptibility" and the latter "differential acclimation." Dr. Child reviews the literature and discusses this subject in some detail in these books: *Senescence and Rejuvenescence* (1915), p. 92; *Individuality in Organisms* (1915), p. 50; *Physiological Foundations of Behavior* (1924), p. 76; also in "The Physiological Gradients," *Protoplasma*, Vol. V (1929), No. 3.

According to Child's theory, if groups of animals have a slower rate of metabolism than single individuals, then differential susceptibility between the slower metabolizing animals of the group and the singles with a higher metabolic rate may play an important rôle in the survival of groups over single individuals in highly toxic solutions. Also, differential acclimation should be effective in weaker solutions and give such singles advantage over the groups. This will be discussed in more detail below.

Fowler (1927) used *Daphnia pulex* de Geer and tested for group protection at different concentrations in the following solutions: calcium chloride, calcium nitrate, magnesium chloride, magnesium nitrate, zinc chloride, zinc nitrate, potassium chloride, potassium hydroxide, potassium cyanide, iron nitrate, barium chloride, barium nitrate, lead chlorate, lead nitrate, aluminum chlorate, aluminum nitrate, aluminum sulphate, sodium chloride, sodium nitrate, sodium nitrite, sodium silicate, sodium hydroxide, nickel sulphate, nickel sulphite, caffeine, ammonium oxalate, silver nitrate, colloidal silver, colloidal sulphur, cupric sulphate, white arsenic, sulphuric acid, nitric acid, and hydrochloric acid. Experiments were made with five different concentrations of each chemical, and the experi-

ment with each concentration was divided into three parts as follows:

1. Twenty animals were distributed, two each, in 20 cc. of the solution in small bottles.

2. Twenty animals were put in 20 cc. of the solution in one small bottle.

3. Twenty animals were put in 200 cc. of the solution in a large bottle.

The survival times were noted, and the pH was checked at the beginning and at the end of the experiments. Results show that pH is not an important factor in these experiments.

When the survival times were tested statistically, those given when 20 animals were crowded into 20 cc. of the solution showed significant group protection over the other two parts of the experiment where the volume per animal was ten times as great. This group protection was consistent at certain concentrations throughout the foregoing list of chemicals. These preliminary experiments did not give us information as to the nature of this group protection. The following experiments are concerned with such an analysis of this phenomenon of group protection of *Daphnia*. They further give the results of an examination concerning whether or not group protection is related to the less widely observed phenomenon of autodestruction as outlined by Drzewina and Bohn. These experiments fall naturally into several groups as different methods are used. Each group of experiments will be described separately, but general discussion of the results will be reserved until all the experiments have been reviewed.

II. EXPERIMENTAL RESULTS

Daphnia longispina were used in all of the following experiments. The chemical solutions were made up in Whitman Laboratory well water except in the cases of sodium hydroxide and potassium hydroxide; distilled water was used for these two chemicals.

1. *Do chemical changes occur as animals live in the solution?*—It was noted above that the most marked protection occurred where the number of animals per unit volume of solution was the greatest. This suggested that animals living in a toxic solution might change

the chemical concentration of the solution so as to render it less toxic. This was tested by using an electrolyte, calcium chloride, as the toxic solution. Experiments were run with six different concentrations of the solution. Each experiment consisted of: (1) 20 animals in 20 cc. of each concentration of the solution, (2) 2 animals in 20 cc. of each concentration of the solution, (3) control solutions in which no animals were present. At the end of the experiments the concentrations of the different solutions were measured by the conductivity method of measuring electrolytes in which a conduc-

TABLE I

SHOWING SUMMARY OF SURVIVAL TIMES AND DIFFERENCES IN RESISTANCES PRODUCED BY *Daphnia* LIVING IN $CaCl_2$ SOLUTION

Molar Concentration of $CaCl_2$	Time in Hours Group Lived	Time in Hours Singles Lived	Resistance of Groups (Ohms)	Resistance of Singles (Ohms)	Resistance of Controls (Ohms)
0.05	62.00	40.84	194.2	191.5	187.0
0.06	51.83	26.66	169.7	168.0	162.5
0.07	26.08	12.24	148.0	148.5	148.25
0.08	11.06	9.66	135.5	125.0	128.5
0.09	9.41	6.74	118.0	117.5	117.7
0.10	5.98	4.16	89.5	91.0	92.0
Totals	166.9	100.3	854.9	841.5	835.95

tivity cell containing the solution to be measured was connected in the circuit of a wheatstone bridge, to which was attached a vibrator; then, with head phones to determine the balancing-point, the resistance of the solution was measured in ohms. The experimental error of this method was about 2 per cent. These experiments were repeated ten times. Table I shows a summary of the results obtained. The data given there show that there was a total resistance of solutions in which groups of animals had lived of 854.9 ohms, as compared with 841.5 ohms for the solutions that contained isolated animals, which indicates that changes have occurred in the former equal to the removal of 1.5 per cent more electrolytes than in the latter. This lies within the experimental error. Further, when the resistances of these solutions are expressed in percentages, and Student's method (1925) for calculating significance of paired experi-

ments is applied to them, the statistical probability is 0.3214, which is well within the range of random sampling and therefore has no significant value. The groups survived a total of 166.9 hours, as compared with 100.3 hours for single individuals. When Student's method is applied to the differences in survival time between groups and single animals in these experiments, the statistical probability is 0.0022. This probability would occur only twenty-two times in ten thousand trials by random sampling, a quite significant proportion.

Another series of experiments were run in exactly the same manner with the single exception that the same solutions were used over and over until the experiment had been repeated ten times. The solutions were then measured by the conductivity method. The total resistance of the solutions of the groups was 51.7 ohms, as compared with 51.6 ohms for the solutions of single animals. Again, this small difference is well within the experimental error and demonstrates that whatever group protection exists cannot be explained on the basis of a reduction of total concentration of electrolytes.

2. *Test for group protection when groups and singles are exposed to the same solution.*—Small tubes, such as were used by De Varigny and Goetsch, were made by fastening fine-mesh cheesecloth over one end of isinglass tubes about $\frac{3}{4}$ inch in diameter. These tubes were fastened together and supported on a wire frame about $\frac{3}{4}$ inch from the bottom of a large crystallization dish, which was filled with 0.072 molar solution of calcium chloride to a depth that would approximate 20 cc. in each tube. Two animals were placed in each of 20 tubes, and 20 animals were placed in each of 10 tubes. The solution in the tubes could diffuse through the cheesecloth with that of the surrounding solution. However, in order to make sure that there was complete mixing of the solution, the tubes were occasionally lifted out of the solution and then lowered back in position. This experiment was repeated four times. Groups showed some survival advantage over single individuals under these conditions, and this advantage was greater when the tubes were allowed to remain for longer intervals before lifting the tubes and thoroughly mixing the solution. This suggests secretion or excretory products as being the influencing agent in the survival advantage of the groups.

3. *Oxygen consumption in groups and single individuals.*—In these experiments on oxygen consumption four different sizes of bottles

were used. The first set ranged from 4.5 cc. to 4.6 cc.; the second from 7.0 cc. to 7.4 cc; the third from 15.9 cc. to 16.9 cc; the fourth from 29.7 cc. to 33.5 cc. These bottles were filled with 0.01 molar solution of calcium chloride. One animal and 5 animals were placed in the first set of bottles; in the second set 3 bottles held respectively 1, 2, and 10 animals; in the third set 1, 4, and 20; and in the fourth and largest set 1, 8, and 40 *Daphnia*. Each set had a control bottle without any animals. In all cases adult animals were used. The bottles were sealed with ground-glass stoppers. The experiments ran for 6 hours except in cases where an animal would die before the end of that time. In such cases the experiment was stopped and oxygen determinations were made at once; as a consequence a few of the experiments ranged between 3 and 6 hours. Most of them, however, ran the full 6 hours. The Winkler method, modified on account of the small volume of sample (Allee, 1929), was used in making oxygen determinations. Table II shows the results of experiments with the first two sizes of bottles. Experiments with the larger bottles in the third and fourth sets gave equivalent results. The amount of oxygen consumed is given in cubic millimeters per animal per hour. In the first set of bottles the average oxygen consumption so expressed is 0.764 for isolated individuals, as compared with 0.414 for the animals composing the groups; in the second set of bottles the averages for 1 animal, 2 animals, and 10 animals are respectively 0.986, 0.646, 0.434; in the third set of bottles the averages for 1, 4, and 20 animals are respectively 0.934, 0.568, 0.459; for the fourth set the averages for 1, 8, and 40 animals are respectively 1.522, 0.696, 0.536 cu. mm.

When these values are expressed in percentages and Student's method for calculating significances is applied to the different groups versus singles, the following figures of statistical probability are obtained:

		Mean Difference	Probability
First set, singles versus 5's		0.350	0.001
Second set	singles versus 2's	.340	.024
	singles versus 10's	.552	.0005
Third set	singles versus 4's	.366	.0018
	singles versus 20's	.375	.0014
Fourth set	singles versus 8's	.916	.002
	singles versus 20's	0.986	0.001

TABLE II

SHOWING DIFFERENCES IN OXYGEN CONSUMPTION BETWEEN GROUPS OF *Daphnia* AND SINGLE INDIVIDUALS IN $CaCl_2$ SOLUTION

APPROXIMATE SIZE OF BOTTLES—4 CC.

Cc. O_2 per Liter, Controls	Cubic Millimeters of Oxygen per Hour per Animal	
	1 Animal	5 Animals
5.35	0.581	0.308
4.91	1.136	.260
7.18	1.358	.602
6.94	0.815	.512
6.94	0.768	.678
6.94	1.023	.603
6.94	0.907	.443
7.29	.684	.597
7.18	.548	.312
7.46	.361	.163
7.88	.605	.439
3.64	0.575	.849
6.25	1.085	.507
6.08	0.645	.272
6.25	0.361	0.166
Av. 6.48	0.764	0.414

APPROXIMATE SIZE OF BOTTLES—8 CC.

Cc. O_2 per Liter, Controls	Cubic Millimeters of Oxygen per Hour per Animal		
	1 Animal	2 Animals	10 Animals
5.52	0.819	0.544	0.360
5.51	0.471	.464	.419
7.19	1.927	.600	.371
7.36	1.089	.848	.733
6.80	1.334	.940	.444
7.31	1.888	.607	.649
7.36	0.260	.970	.598
7.54	0.509	.520	.461
7.71	1.190	.777	.448
7.96	0.800	.411	.301
8.04	0.652	0.520	.416
4.65	1.887	1.110	.460
5.82	0.549	0.350	.378
6.70	1.093	.677	.406
6.82	0.328	0.351	0.075
Av. 6.83	0.986	0.646	0.434

These figures are all of clearly significant value and indicate that the decrease in oxygen consumption shown by grouped as compared with single individuals is not due to chance of random sampling.

In order to avoid any criticism of the foregoing data on the basis that the oxygen consumption of a single animal was compared with the mean of the group, another set of experiments was made, using the third set of bottles in which the number of single individuals was equal to those of the group (15) animals. Then, by comparing the mean oxygen consumption of the group, which was 0.8124 cu. mm., with the mean of the singles, which was 1.3211 cu. mm. per animal per hour, and calculating the statistical significance as stated above, the figure of probability is 0.0001, which compares favorably with the probability figures just given. Accordingly, we may conclude that grouped animals use less oxygen per individual than do single individuals.

4. *Influence of unaërated conditioned solution upon oxygen consumption in single individuals.*—These experiments were arranged similarly to those in Section 3 except the solution in which singles were placed had been homotypically conditioned by having groups of *Daphnia* live in it. Also, the bottles with intermediate-sized groups were eliminated in these experiments, and tests were made with singles and large groups only. Table III shows the results of a series of seven experiments with the first and second sets of bottles. The third set of bottles gave equivalent results. The largest set of bottles was not used in these experiments. In the first set of bottles the average oxygen consumption expressed in cubic millimeters per animal per hour is 0.579 for single individuals, as compared with 0.538 for the groups; for the second set of bottles the average for single animals is 0.616, as compared with 0.575 for groups of 10 animals; in the third set of bottles the average for singles is 0.693, as compared with 0.694 for groups of 20 animals.

When these results were treated statistically by Student's method, the following figures of probability are obtained:

	Mean Difference	Probability
First set, singles versus 5's	0.041	0.570
Second set, singles versus 10's	.041	.759
Third set, singles versus 20's	0.001	0.807

These figures of probability are clearly not significant, and indicate that single individuals in conditioned solution have their rate of metabolism lowered to that of the group in raw solution. These results suggest that the difference in metabolism, as measured by

TABLE III

SHOWING EFFECTS OF UNAËRATED CONDITIONED SOLUTION UPON OXYGEN CONSUMPTION IN *Daphnia*

APPROXIMATE SIZE OF BOTTLES—4 CC.

Oxygen Tension in Unaërated Conditioned Solution	1 Animal (Cubic Millimeters of Oxygen per Animal per Hour)	Oxygen Tension in Raw Solution	5 Animals (Cubic Millimeters of Oxygen per Animal per Hour)
4.76	0.787	6.23	0.447
4.15	1.058	7.28	.668
3.45	0.364	5.45	.553
4.08	.453	5.84	.649
4.26	.544	5.45	.507
4.07	.364	6.88	.577
4.36	0.453	5.31	0.363
Av. 4.16	0.579	6.06	0.538

APPROXIMATE SIZE OF BOTTLES—8 CC.

Oxygen Tension in Unaërated Conditioned Solution	1 Animal (Cubic Millimeters of Oxygen per Animal per Hour)	Oxygen Tension in Raw Solution	10 Animals (Cubic Millimeters of Oxygen per Animal per Hour)
5.90	0.512	6.31	0.554
3.25	.654	6.52	.635
3.01	.727	6.46	.608
3.68	.362	6.00	.599
4.06	.860	5.44	.490
4.01	.543	6.15	.596
4.54	0.653	5.85	0.545
Av. 4.06	0.616	6.10	0.575

oxygen consumption, between single individuals and groups of animals is due to the accumulation of waste products in the medium of the group, or that it is due to the difference in oxygen tension between the two solutions, which at the beginning of the experiments was only 66 per cent of air saturation in the conditioned solution.

5. *Influence of aërated conditioned solution upon oxygen consumption in single individuals.*—This series of experiments were run ex-

actly as were those in Section 4 except the conditioned solution was aërated before the single animals were put into it. The results are quite different from those in Section 4 and agree very closely with those in Section 3. In the first set of bottles the average oxygen consumption, expressed in cubic millimeters per animal per hour, is 0.617 for single individuals, as compared with 0.433 for the groups; for the second set of bottles the average for the single animals is 0.813, as compared with 0.512 for groups of 10 animals; for the third set of bottles the average for singles is 0.901, as compared with 0.604 for groups of 20 animals.

When these results are treated statistically by Student's method, the following figures of probability are obtained:

	Mean Difference	Probability
First set, singles versus 5's	0.184	0.009
Second set, singles versus 10's	.301	.011
Third set, singles versus 20's	0.297	0.0098

These figures of probability are all significant. These results indicate that the substance or substances which retards metabolism in *Daphnia* when they are crowded into a small volume is of a volatile nature and is eliminated by aërating the solution, or that the increased metabolism of the singles in these aërated, conditioned solutions, as compared with those in unaërated conditioned solutions, may be due to increasing the oxygen tension. In these experiments the oxygen tension of the conditioned solution was about 80 per cent of air saturation.

6. *Influence of reduced oxygen tension upon the rate of oxygen consumption in Daphnia.*—A series of 10 experiments were made to determine the effects of reduced oxygen tension on the rate of oxygen consumption in *Daphnia;* 20 cc. bottles were used in these experiments. In one part of each experiment 5 animals were put in each bottle with oxygen tension at air saturation in 0.01 molar solution of calcium chloride. In the second part of the experiment 5 animals were put in each bottle of the same solution which had its oxygen tension reduced to about 50 per cent of air saturation by bubbling nitrogen through it. The experiments ran for 6 hours, at

the end of which time oxygen determinations were made with the modified Winkler method. Table IV shows the oxygen tensions at the beginning and at the end of the experiments, with the amount of

TABLE IV

SHOWING EFFECTS OF REDUCED OXYGEN TENSION UPON THE RATE OF OXYGEN CONSUMPTION IN *Daphnia*

APPROXIMATE SIZE OF BOTTLES—20 CC.

Normal Oxygen Tension, Control	Oxygen Tension at End of Experiment	5 Animals (Cubic Millimeters of Oxygen per Animal per Hour)
6.79	5.74	0.678
7.06	6.16	.547
7.41	6.16	.765
7.56	6.53	.774
7.62	6.65	.548
7.65	6.31	.690
7.64	6.52	.682
7.56	6.16	.864
6.98	5.48	0.913
6.98	5.12	1.123
Av. 7.33	6.08	0.758

APPROXIMATE SIZE OF BOTTLES—20 CC.

Reduced Oxygen Tension, Control	Oxygen Tension at End of Experiment	5 Animals (Cubic Millimeters of Oxygen per Animal per Hour)
4.39	3.28	0.750
5.67	4.86	.598
4.12	3.13	.679
3.02	2.50	.382
3.08	2.46	.414
3.49	2.36	.763
3.91	2.84	0.712
4.11	2.44	1.099
4.32	2.98	0.916
4.32	3.82	0.507
Av. 4.04	3.00	0.682

oxygen consumed per animal per hour during the experiments. On the average, the animals in the solution with oxygen tension at air saturation consumed 0.758 cu. mm. per animal per hour, as compared with 0.682 cu. mm. for the animals living in the same solution with oxygen tension reduced to 50 per cent of air saturation. When the differences in oxygen consumption in the two solutions

are tested statistically by Student's method, the figure of probability is 0.322, which is clearly not significant. This indicates that a difference in oxygen tension of 50 per cent of air saturation does not influence oxygen consumption in *Daphnia* sufficiently to be detected by the method used. However, when this series of experiments was repeated, using 8 cc. bottles with 2 animals in each, the average oxygen consumption in the air-saturated solution was 0.840 cu. mm. per animal per hour, as compared with 0.639 cu. mm. in the solution with oxygen tension reduced to 50 per cent of air saturation. The statistical probability in this case is 0.015, a significant value. Between these two sets of conflicting data the former should be given more weight because the experimental error in oxygen determinations was smaller in the larger volumes and larger numbers of animals. Since the differences in oxygen tension in these experiments are equal to or greater than the differences between groups and singles at the end of the experiments in Section 3 rather than at their beginning, it seems safe to assume that differences in oxygen tension have played at most only a minor part in causing differences in oxygen consumption in these experiments.

7. *The effects of carbon dioxide upon oxygen consumption in Daphnia.*—Three series of experiments were run, using 0.005 molar solution of potassium chloride as the toxic agent and 20 cc. bottles with ground-glass stoppers as containers. Each series of experiments consisted of two parts. The first part had 4 bottles with 20 animals in each in the KCl solution. The second part had 16 bottles with 2 animals in each in the same-strength solution which had previously had carbon dioxide bubbled slowly through it for 5 minutes. At the end of 6 hours oxygen determinations were made. Table V shows a summary of these results. In the bottles where 2 animals were present, the average oxygen consumption was 0.275 cu. mm. per animal per hour, as compared with 0.465 cu. mm. where 20 animals were present in the solution that had no carbon dioxide bubbled through it. When these results are treated statistically by Fisher's method of comparing means (Fisher, 1928, p. 107), the figure of probability is 0.0018, a quite significant value. These results indicate the importance of carbon dioxide as a retarding factor in metabolism of *Daphnia*.

8. *Effects of removing carbon dioxide from a 0.01 molar solution of calcium chloride upon the survival time of Daphnia living in it.*—

Watch glasses were used in these experiments instead of bottles

TABLE V

SHOWING EFFECTS OF CO_2 ON RATE OF OXYGEN CONSUMPTION IN *Daphnia*

Oxygen Tension in 0.005 Molar Solution KCl	20-Animal Groups (Mean Cubic Millimeters of Oxygen per Animal per Hour)	Oxygen Tension of 0.005 KCl Solution after CO_2 Was Bubbled Through	2 Animals, in CO_2 Bottles (Mean Cubic Millimeters of Oxygen per Animal per Hour)
8.0	0.482	6.65	0.240
8.2	.457	6.71	.261
8.0	0.457	6.61	0.323

so that more surface would be exposed. Ten cubic centimeters of 0.01 molar solution of calcium chloride were put in each watch glass. Five animals were put in each of 4 of the watch glasses, and single

TABLE VI

SHOWING EFFECTS OF REMOVING CO_2 SOLUTION UPON THE SURVIVAL TIME OF *Daphnia* LIVING IN IT

Exp. No.	Time in Hours	Number Surviving in 2 Groups of 5 Animals	NaOH, Number Surviving in 2 Groups of 5 Animals	Number Surviving in Singles, 10 Animals	NaOH, Number Surviving in Singles, 10 Animals
1	6	7	3	8	3
2	11	6	3	6	1
3	11	10	6	10	5
4	8	6	2	6	2
5	5	6	1	7	1
6	6	9	3	8	4
7	13	10	4	8	5
8	8	10	2	10	3
9	8	7	4	6	2
10	8	10	2	9	2
Total....	.....	81	30	78	28

individuals in each of 20 of them. One-half of these, two groups of 5 and 10 singles, were sealed in a desiccator which had a strong solution of sodium hydroxide in its bottom chamber. The other half of the experiment was sealed in a desiccator without NaOH solution. Survival times of all *Daphnia* were carefully noted. Table VI shows

the results of these experiments. At the end of the experiment 81 of the group animals in the desiccator without NaOH solution were still surviving, as compared with 30 animals in the desiccator with NaOH solution. Likewise, 78 of the single animals were still surviving in the desiccator without NaOH solution, while only 28 singles were surviving in the desiccator with NaOH solution. When these results are treated statistically by Student's method, the following figures of probability are obtained:

	Probability
Groups in NaOH desiccator versus groups in other desiccator.....	0.0001—
Singles in NaOH desiccator versus singles in other desiccator.....	0.0001—

The minus signs after the probability figures indicate that the probabilities are less than those covered in Fisher's tables. These results show that the presence of carbon dioxide does influence the survival time of *Daphnia* living in a toxic solution.

All the evidence thus far indicates that the survival advantage of groups of *Daphnia* over that of single individuals when exposed to toxic solutions is due to slower rate of metabolism of the group, brought about largely by the accumulation of carbon dioxide in the limited volume of the group. This survival advantage of groups of *Daphnia* falls in line with Dr. Child's theory of differential susceptibility and agrees with his numerous demonstrations that various animals, when exposed to a large variety of injurious agents, survive longest in strong solutions when their rate of metabolism is slowest. The question naturally arises whether or not there is also differential acclimation between groups and single individuals in more dilute concentrations of toxic solutions where death does not occur too soon for some acclimation to take place.

In checking over all my available data, it was noted that the few times that singles lived longer than groups occurred in the more dilute concentrations of the toxic solutions. In order to test this point in more detail, a variety of chemicals were used and a series of survival experiments were made at different concentrations of each chemical solution to determine if there are concentrations in which single individuals will live longer than groups.

9. *Survival experiments with different concentrations of calcium-chloride solution.*—A series of 8 experiments were made with each of the following concentrations of molar solutions of calcium chloride: 0.025, 0.0375, 0.05, 0.065. Each experiment consisted of one group of 20 animals in 20 cc. of the solution in a half-ounce bottle, and 20 single animals one each in 20 cc. of the solution in a half-ounce bottle. Table VII shows the results of two concentrations: 0.0375

TABLE VII

SHOWING DIFFERENTIAL SURVIVAL BETWEEN GROUPS AND SINGLE *Daphnia* IN DIFFERENT CONCENTRATIONS OF $CaCl_2$ SOLUTION

0.0375 MOLAR SOLUTION $CaCl_2$ TIME, 10 HOURS		0.065 MOLAR SOLUTION $CaCl_2$ TIME, 7 HOURS	
Number Surviving in Singles	Number Surviving in Groups	Number Surviving in Groups	Number Surviving in Singles
6	1	4	1
4	1	3	0
5	1	7	0
7	2	5	1
7	2	5	0
7	2	2	0
9	5	3	1
12	6	5	0
57	20	34	3

and 0.065. In the 0.065 solution there were 34 of the groups living at the end of the experiment and 3 of the singles. In the 0.0375 solution there were 57 of the singles living, as compared with 20 of the groups. In the 0.05 solution there were 36 of the singles surviving, while only 7 of the groups were living. In the 0.025 solution there were 103 of the singles living, as compared with 46 of the groups. When Student's method is applied to these data, the figures of probability are:

	Probability
In 0.065 solution.....	0.0001 group advantage
In 0.05 solution.....	.016 single advantage
In 0.375 solution.....	.0001 single advantage
In 0.025 solution.....	0.001 single advantage

These figures of probability are all significant.

Table VIII shows a summary of the results of other chemicals treated similar to calcium chloride. In all these chemical solutions except sodium hydroxide and potassium hydroxide the groups survived better than singles in the more concentrated solution, and single individuals survived better than groups in more dilute solutions. In NaOH and KOH solutions the groups survived better than single individuals in all the concentrations tested. The results with

TABLE VIII

SHOWING SUMMARY OF OTHER CHEMICALS TREATED AS $CaCl_2$ IN TABLE VII

Chemical	Molar Concentration	Time in Hours	Probability, Group Advantage	Probability, Single Advantage
KCl	0.05	1.5	0.0008	
KCl	.025	6 75	.....	0.002
$MgCl_2$	.05	18.0	.002	
$MgCl_2$	.037	43.0	.....	.0001—
$Mg(SO_4)_2$	.2	6.0	.0001	
$Mg(SO_4)_2$	.15	11.0	.....	.005
$Ca(NO_3)_2$	.05	3.5	.0008	
$Ca(NO_3)_2$	.03	8.5	.....	.0001—
$Zn(NO_3)_2$	.015	3.5	.0001—	
$Zn(NO_3)_2$	.001	15.0	.....	.0008
$NaNO_3$	.135	1.2	.0001—	
$NaNO_3$	.075	36.0	.....	.0001—
NaCl	.15	1.25	.0001—	
NaCl	.05	66.0	.....	0.0001—
NaOH	.005	1.25	.002	
NaOH	.0006	28.0	.0001—	
KOH	.0025	2.0	.0014	
KOH	0.00015	36.0	0.0001	

sodium hydroxide and potassium hydroxide suggest that carbon dioxide produced by the animals reacts with the hydroxide solutions to form carbonates and water, thus making the hydroxide solutions more dilute; and since this process takes place more rapidly, other conditions being equal, the larger the numbers of animals present, the groups are able to survive longer than single individuals.

10. *Change in concentration of hydroxide solutions due to animals living in it.*—A large volume of dilute solution of sodium hydroxide was made in distilled water. From this stock solution 20 cc. were put in a number of half-ounce bottles. Some of these bottles were kept for controls without any animals in them. Two animals were put in each of 8 bottles, and 20 animals were put in each of 8 of

them. All were kept under identical conditions. After 3 hours the concentrations of these solutions were determined by titrating against hydrogen chloride with a known factor and using phenolphthalein and methyl orange as indicators. Table IX shows the results of these experiments. The mean molar concentration of the controls at the end of the experiments was 0.00094; this had been reduced to 0.000764 molar where 2 animals were present, and to 0.000606 molar when 20 were present. When the results of these experiments were expressed in percentages and treated statistically by Fisher's method

TABLE IX

SHOWING CHANGES IN NaOH SOLUTION DUE TO *Daphnia* LIVING IN IT

CUBIC CENTIMETERS OF NaOH SOLUTION	TIME IN HOURS	MOLAR CONCENTRATION		
		Control	2 Animals	20 Animals
20	3	0.000974	0.000575	0.000575
20	3	.000936	.000755	.000589
20	3	.000950	.000755	.000625
20	3	.000894	.000800	.000625
20	3	.000980	.000838	.000650
20	3	.000949	.000813	.000581
20	3	.000894	.000769	.000625
20	3	0.000940	0.000806	0.000581
Mean.....	.....	0.000940	0.000764	0.000606

for comparing means, the figure of probability is 0.0022. Identical experiments with dilute solution of potassium hydroxide gave the same type of results as in NaOH solution. The figure of probability in this case was 0.0001. Both of these figures of probability are quite significant, and the experiments show that groups of animals living in these solutions change the concentrations significantly over that of single individuals. These results give a satisfactory explanation for groups being able to survive longer than single individuals throughout the different concentrations of NaOH and KOH solutions.

11. *Effects of introducing carbon dioxide into a solution of calcium chloride upon the survival time of Daphnia living in it.*—The two concentrations of calcium chloride chosen for these experiments were the same as those which had been used in Section 9; at that time in

the 0.065 molar solution, groups survived longer than single individuals; in the other concentration, 0.025 molar solution, single individuals lived longer than the groups. Two sets of experiments were made with each of these concentrations. A large volume of 0.065 molar solution of calcium chloride was prepared. This was divided into two parts. From one part a set of experiments were made by putting 20 cc. of the solution into each of 88 half-ounce bottles. Twenty animals were put into each of 8 of these bottles, and 2 animals were put into each of 80 of them, making an initial number of 320 *Daphnia* equally divided between the two population densities. These were then paired off so that one group of 20 would be checked with 10 of the bottles containing 2 animals each. The other part of the solution had carbon dioxide bubbled slowly through it for 5 minutes; then an identical set of experiments were prepared as described above for the first part of the solution. These two sets of experiments were run simultaneously and under approximately the same environmental conditions. The survival times of all these were carefully checked. Table X shows the results of these experiments at definite times from which statistical calculations were made by applying Student's method.

Two sets of similar experiments were made with 0.025 molar solution of calcium chloride. Table XI shows the results obtained. When Student's method was applied to the results of the experiments with these two concentrations of calcium chloride, the following statistical probabilities were obtained:

At the end of 1 hour in the control 0.065 molar solution of calcium chloride, 76 of the groups were surviving, as compared with 4 of the single individuals; group advantage is indicated in this case with a probability figure of 0.0001. At the end of 2 hours in the 0.065 molar solution which had carbon dioxide bubbled through it, there were 62 of the grouped *Daphnia* surviving, as compared with 20 of the single individuals; the figure of the probability in this case is also 0.0001. At the end of 1.5 hours in the 0.065 molar solution, there were 10 animals of the groups living, as compared with 20 of the single individuals at the end of 2 hours in the 0.065 molar solution which had CO_2 bubbled through it; singles have a survival advantage in this case with a probability figure of 0.0016. At the end

of 30 hours in the 0.025 molar solution, there were 132 single animals living, as compared with 70 animals of the groups; singles have a survival advantage in this case with a probability of 0.0075. At the end of 27 hours in the 0.025 molar solution which had CO_2, bubbled

TABLE X

SHOWING EFFECTS OF CO_2 UPON THE SURVIVAL TIME OF *Daphnia* IN 0.065 $CaCl_2$ SOLUTION

0.065 MOLAR SOLUTION $CaCl_2$			
Time, 1 Hour		Time, 15 Hours	
Groups Surviving	Singles Surviving	Groups Surviving	Singles Surviving
10	2	2	0
10	1	2	0
10	0	1	0
10	0	1	0
8	0	0	0
10	1	3	0
9	0	0	0
9	0	1	0
76	4	10	0

CO_2 BUBBLED FOR 5 MINUTES THROUGH 0.065 $CaCl_2$ SOLUTION			
Time, 2 Hours			
Groups Surviving	Singles Surviving	Groups Surviving	Singles Surviving
10	3	8	5
10	3	5	1
6	3	10	1
5	3		
8	1	62	20

through it, 18 animals of the groups were surviving, as compared with 20 of the single animals; the probability figure in this case is 0.5006, which is not significant. At the end of 30 hours in the 0.025 molar solution without CO_2, there were 70 animals of the groups surviving, as compared with 18 single animals in the same solution, which had CO_2 bubbled through it, at the end of 27 hours.

An analysis of the foregoing data shows:

1. That the results of these experiments when CO_2 was not

bubbled through the solution agree with previous results of the same concentrations.

2. That bubbling CO_2 through 0.025 molar solution of calcium chloride destroys the normal survival advantage which singles have over groups at this concentration.

3. That bubbling CO_2 through 0.065 molar solution makes it possible for singles to survive longer than groups in the same concentration when no CO_2 has been bubbled through it, whereas groups normally live longer than singles at this concentration.

TABLE XI

SHOWING EFFECTS OF CO_2 UPON THE SURVIVAL TIME OF *Daphnia* IN 0.025 $CaCl_2$ SOLUTION

0.025 MOLAR SOLUTION $CaCl_2$		CO_2 BUBBLED 5 MINUTES THROUGH 0.025 MOLAR SOLUTION $CaCl_2$	
Time, 30 Hours		Time, 27 Hours	
Groups Surviving	Singles Surviving	Groups Surviving	Singles Surviving
10	18	2	1
5	18	2	3
12	15	3	3
12	15	2	3
4	18	1	3
10	15	4	3
15	15	3	2
2	18	1	2
70	132	18	20

4. That groups in 0.025 molar solution survive longer than singles in the same solution which has had CO_2 bubbled through it; whereas singles normally survive longer than groups at this concentration. In other words, the introduction of CO_2 into a solution where single individuals are living produces the same type of effect which is produced by groups of animals living in a small volume.

III. DISCUSSION AND CONCLUSIONS

There have been several theories postulated to explain the widespread phenomenon of differential survival of groups over that of single individuals when placed into a limited volume of injurious

medium. These theories are briefly stated in the Introduction. Each theory has data to support it with the materials and under the conditions in which the experiments were made. It is reasonable to suppose that there is no one factor that will explain all this phenomenon of group protection as it occurs among phylogenetically widely differing groups of animals and under diverse conditions. It is probable that there are several factors working to produce group protection in each case where it has been observed, with perhaps one factor predominating in effect among one group of animals under certain conditions and a different factor predominating with a different group of animals under different conditions. The question which concerns us most here is: Which factor or factors have been the most influential in causing differential survival between groups and single individuals among *Daphnia* in these experiments?

Allee and Schuett (1927) showed that groups of *Planaria*, *Ophiderma*, and other aquatic animals lived longer than single individuals in colloidal silver because the groups produced slime in greater quantities than single individuals or otherwise adsorbed the colloidal silver and rendered the solution less toxic to animals comprising the group. Fowler (1927) showed that groups of *Daphnia* lived longer than single individuals in the same volume and concentration of toxic solution of numerous chemicals, chiefly electrolytes. In both cases the amount of chemical per animal would be much less in the group. The question as to whether groups of *Daphnia* living in a toxic solution weaken it more than would single individuals was tested by experiments recorded in Section 1 by the conductivity method of measuring concentrations of electrolytes. The results show that the concentration of an electrolyte, calcium chloride, was not changed significantly by the *Daphnia* living in it. If there are chemical reactions between the products of the *Daphnia* and their chemical medium, in so far as changes could be detected by the method used, the end-results of such reactions leave the medium with approximately the same degree of ionization.

For calcium chloride and presumably for other similar electrolytes, we have certainly not proved that with these animals chemical change of the medium is a major factor in group protection. On the other hand, in Section 10 data are given which demonstrate

clearly that, when exposed to sodium or potassum hydroxide, *Daphnia* do alter the chemical composition of the solution, the more rapidly, other things being equal, the greater the number of animals present. Under the adverse conditions furnished by these alkalies, group protection is due to the removal of the toxic material as a result of the more rapid production of metabolic products (in this case carbon dioxide) by the group. Preliminary tests with the conductivity method did not detect the chemical changes which occur in the hydroxide solutions due to animals living in them, which means that carbonate ions were substituted for the hydroxyl ions in these solutions. However, this type of reaction does not seem to hold for the salts which were tested with the conductivity method in the foregoing experiments, because in the first place the affinity between the metals and chlorine to form salts is greater than that between the metal and carbon dioxide to form carbonates. Secondly, the fact that groups lived longer in strong concentrations of the salts than did single individuals, while single animals lived longer than groups in weaker concentrations, indicates that the effects of carbon dioxide were upon the animals instead of reacting with the chemical medium, as in the cases with hydroxides, where the groups survived longer than single individuals throughout all the concentrations tested.

The results of the experiments with tubes having bottoms of cheesecloth (Section 2) where the groups and single individuals were kept in the same solution but limited in their environment indicate that there is an influence of some kind which gives advantage to the groups. These results show also that this influence can be eliminated almost, if not entirely, by frequently lifting the tubes out of the solution and thoroughly mixing the medium. These experiments do not make clear what this influence is. It may be the result of contact among members of the group, which Bilski and Goetsch showed leave an adverse effect upon survival or which Allee (1927) and Drzewina and Bohn (1928) report has positive survival value under certain conditions. It may also be the result of secretions (Drzewina and Bohn, 1921); or it may be that excretory products of the group or the lessened oxygen tension brought about by the group influences their metabolic rate and hence causes differential survival.

Further studies of the influence of grouping animals together are reported in Section 3, in which oxygen consumption is measured. These results show a marked reduction in the rate of oxygen consumption of the group as compared with singles. This means that metabolism has been greatly reduced in the individuals composing the groups, due probably to one or more of the factors given in the preceding paragraph. Experiments with aërated conditioned solution and raw solution of calcium chloride (Section 4, Table III) throw some light on the subject. Since single individuals living in unaërated conditioned solution consume oxygen no faster than the groups in raw solution of the same concentration, it is evident that the causes for retarding metabolism in groups are retained in the solution after the animals are removed.

Experiments with aërated conditioned solution and raw solutions of calcium chloride (Section 5) throw further light on the subject. Since single individuals living in aërated conditioned solutions do not have their metabolism retarded and consume oxygen much faster than groups in raw solution of the same concentration, it is evident that factors retarding metabolism among groups of *Daphnia* are of a volatile nature and are eliminated from a solution by aërating it or result from low oxygen tension. This suggests that the metabolic rate of groups is retarded either by accumulation of carbon dioxide which is given off into the medium in greater quantities by the group or by reduced oxygen tension in the medium of the group. The results of experiments upon the effects of reducing oxygen tension in calcium chloride solution to about 50 per cent of air saturation upon the rate of oxygen consumption of animals living in it tend to minimize the importance of this factor in these experiments (Section 6, Table IV). The larger numbers of animals in the larger-sized bottles show no significant difference in oxygen consumption between animals living in a solution of air saturation and animals living in the same concentration of calcium chloride with the initial oxygen tension at 50 per cent of air saturation. However, the results obtained from an analysis based on the exposure of smaller number of animals in smaller bottles show exactly the opposite results. The former set of data should be given more weight on account of experimental error being considerably less with the larger numbers of

animals in larger volumes of solution. This conclusion that oxygen tension is not an important factor in group protection is further strengthened by the fact that the usual survival experiments were run in uncorked wide-mouth bottles which allowed continuous aëration. Still, the results in the latter case must be considered as indicative that reduced oxygen tension may play a rôle in retarding metabolism among groups of *Daphnia* in a limited volume which is not exposed to the air. An examination of the data in the foregoing experiments indicates that it is very improbable that differences in oxygen tension even in closed vessels have been an important factor in reducing metabolism, because the rate of metabolism was reduced relatively more in the groups of intermediate size (Section 3) than in the largest groups, where oxygen tension was most reduced. When oxygen consumption of these intermediate-sized groups is compared with that of single individuals in the same concentration of calcium chloride, the rate is significantly lower among the groups even when the oxygen tension is 85 per cent of air saturation at the end of the experiment. On the other hand, single individuals (Section 5) introduced into an aërated conditioned solution of calcium chloride with an initial oxygen tension at 80 per cent of air saturation did not have their rate of oxygen consumption appreciably lowered as compared with that shown by groups of animals in raw $CaCl_2$ solution of the same concentration with oxygen tension at air saturation. Since groups of intermediate numbers have their rate of metabolism significantly lowered when oxygen tension in 85 per cent of air saturation at the end of the experiment, while single individuals do not have their rate of metabolism appreciably lowered even when oxygen tension is 80 per cent of air saturation at the beginning of the experiment, it seems safe to assume that differences in oxygen tension have played, at most, only a minor rôle in reducing the rate of metabolism among groups of animals in these experiments. These conclusions agree, on the whole, with the results of many recent investigations which show that the rate of oxygen consumption among animals is independent of oxygen tension down to about 50 per cent or less of air saturation (Lund, 1921; Garrett, 1928; Helff, 1929).

The other factor indicated as possibly influential in reducing the rate of metabolism of groups of *Daphnia* is the accumulation of

carbon dioxide in their medium, which is produced in larger quantities by groups of animals than by single individuals. It is interesting to note that introducing carbon dioxide into a solution of potassium chloride (Section 6, Table V) markedly lowers the rate of metabolism of animals living in it. Even the rate of oxygen consumption of single individuals is reduced to about one-half that of groups living in the same concentration of potassium chloride when no carbon dioxide has been added; whereas groups in previous experiments consumed only about one-half as much oxygen as single individuals. It is evident here, also, that oxygen tension plays little or no part in reducing the rate of metabolism, because the groups in these experiments have the same reduced oxygen tension as do others discussed in Section 3, where single individuals consumed oxygen much faster than groups. These results give us ample evidence that carbon dioxide is a very important factor in reducing the rate of metabolism in grouped *Daphnia*, and it is very probable that accumulation of carbon dioxide has been the most important factor in reducing the rate of metabolism of groups in all these experiments.

When carbon dioxide is removed from a solution in which animals are living (Section 8, Table VI), their survival times are greatly reduced. It appears again from these results that accumulation of carbon dioxide among groups of *Daphnia* may be a very important factor in causing group protection by slowing down their rate of metabolism and thus, through differential susceptibility, allowing groups to survive longer than single individuals (Child, 1915, 1924). This makes it possible to explain group protection in *Daphnia* exposed to relatively highly toxic solution in terms of Child's theory of differential susceptibility. If this is true, then according to the other part of this theory there should be differential acclimation in the weaker concentrations of toxic solutions. Under such conditions single individuals, which have a higher rate of metabolism than do those in groups, should survive longer than groups, thus giving the type of results which Drzewina and Bohn (1921–22) ascribe to an autodestructive secretion in the cases of *Polycelis* and *Convoluta* in KCl solutions.

Experiments reported in Section 9, Tables VII and VIII, give considerable data on this subject covering a number of different

chemical solutions. These data demonstrate differential susceptibility and differential acclimation between groups and single individuals in all cases except in NaOH and KOH solutions. In these two solutions there is differential susceptibility only, that is, groups survived longer than singles in all cases tested in these solutions. The experiments with NaOH and KOH solutions (Section 10, Table IX) furnish data to show that carbon dioxide reacts with the solutions to form carbonates and water, thus weakening the toxic concentration of the medium and permitting the groups to survive longer than the single individuals. Under these conditions differential acclimation would not take place.

Further evidence that CO_2 is an important factor in producing differential susceptibility and differential acclimation is obtained by introducing carbon dioxide into a strong solution of calcium chloride where single animals are living. One can increase the survival time of the singles until they live significantly longer than groups in the same concentration of calcium chloride. In more dilute solutions of calcium chloride, where singles ordinarily survive longer than groups, one can use the same method and decrease the survival time of the singles until the groups survive significantly longer (Section 11, Tables X and XI). In other words, by varying the amount of carbon dioxide in a solution of calcium chloride, it is possible to reverse differential susceptibility and differential acclimation between groups of *Daphnia* and single individuals.

After considering all the data at hand, it seems safe to conclude that the differential rate of accumulation of carbon dioxide in the medium is the most important factor in influencing differential survival between groups of *Daphnia* and single individuals in these experiments. These results do not disprove other theories of group protection with other animals or even with *Daphnia* under different conditions. They do demonstrate a hitherto neglected factor which must be considered when survival time between groups of animals and single individuals is being studied.

This type of differential survival between groups and single individuals may be of considerable biological importance to *Daphnia*, furnishing, as it does, two survival mechanisms, one of which is suitable for a sparse population in a mildly injurious environment,

while the other acts when many *Daphnia* are crowded together in the presence of more extreme toxicity. Under these conditions some members of the race have a chance of survival if the toxicity be not too great or of too long duration.

IV. SUMMARY

1. *Daphnia* living in a $CaCl_2$ solution do not alter its electrical conductivity to any appreciable extent.

2. Numbers of *Daphnia* living in a limited volume of $CaCl_2$ solution consume significantly less oxygen than single individuals living in the same concentration of this electrolyte.

3. Single individuals living in unaërated conditioned solutions of calcium chloride have their rate of oxygen consumption lowered to that of groups in the same concentration of raw solution.

4. Single individuals living in aërated conditioned solutions of calcium chloride do not have their rate of metabolism lowered to any appreciable extent.

5. Reduced oxygen tension has very little influence on oxygen consumption in *Daphnia*, at least down to 50 per cent of air saturation.

6. Diffusion of carbon dioxide into a solution markedly lowers the rate of oxygen consumption of *Daphnia* living in that solution.

7. Removal of carbon dioxide from a solution of calcium chloride significantly shortens the survival time of *Daphnia* living in it.

8. Groups of *Daphnia* survive longer than single individuals in stronger concentrations of a variety of toxic solutions, while single individuals survive longer than groups in weaker concentrations of the same solutions.

9. *Daphnia* living in dilute solutions of sodium hydroxide and potassium hydroxide significantly weaken the concentration of these chemicals.

10. Introducing carbon dioxide into toxic solutions where *Daphnia* are living increases their survival time in strong concentrations and decreases their survival time in weak concentrations.

11. Differential survival between groups of *Daphnia* and single individuals can be explained on the basis of Child's theory of differential susceptibility and differential acclimation of slowly and rapidly metabolizing animals.

12. All these data indicate that carbon dioxide is a very important factor in causing differential survival between groups of *Daphnia* and single individuals when exposed to toxic solutions.

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